d) Remarks

Claims 1-8 remain pending in the case, claims 1 and 2 having been amended above. The purpose of the amendments is to clarify the claim language for ease of comprehension. The amendments are not narrowing in scope, rather they merely restate the originally claimed subject matter in a more understandable manner, to wit:

> The term "sample" has been replaced with "environment" to clarify that the invention is for testing for the presence of an analyte in any surrounding where the analyte is potentially present, such as in water or air. Support for the amendment is found in the specification on p. 5, lines 6-7 and p. 13, lines 4-6, among other places. It is believed that the term "sample" might be misconstrued to mean that the invention is applied to a sample of water or air removed from the environment; however, though the invention could be employed in such a manner, it is obviously not so limited.
>
> Subpart (a) of claim 1 has been amended to make explicit what was inherent in the original claim, that is, that the binding protein has an active site "at which the analyte will bind if present" and that the colorimetric indicator and binding protein "form a complex". Support for the amendments is found in the specification on p. 8, line 20 - p. 9, line 1 and p. 7, lines 9-11, among other places. A corresponding amendment has been made to claim 2.
>
> Subpart (b) of claim 1 has been amended to more precisely recite that the "complex" is exposed to "said environment". While the language as originally presented (that the colorimetric indicator and binding protein are exposed) is technically accurate, at the time of exposure the two are complexed. The amendment makes the claim easier to follow and understand.
>
> Wording from subparts (c) and (d) have been re-arranged to better emphasize the measuring and determining steps. Like the amendment to subpart (b), these amendments make the claim easier to follow and understand. The amendments also make explicit that it is a spectral value of colorimetric indicator that is measured and, from this, a determination is made as to the presence of the analyte of interest.

Claims 1-4 stand rejected under 35 USC 102(b) as being anticipated by Iwasa et al., U.S. Patent No. 4,517,290. Claim 5 stands rejected under 35 USC 103(a) as being unpatentable over Iwasa et al. in view of Khabashesku et al., U.S. Patent No. 6,428,762. Claims 6-8 stand rejected under 103(a) as being unpatentable over Iwasa et al. in view of Chapoteau et al., U.S. Patent No. 5,262,330.

The examiner's rejections are respectfully traversed.

General Remarks Regarding Iwasa et al.

The Iwasa et al. patent deals with an enzyme immunoassay technique for the detection of peptide antigens such as hormones. The detection technique is an antibody "sandwich" system that works as follows.

Antibodies (Ab) against the antigen are generated by conventional means of injecting the purified antigens into animals, obtaining the Ab-containing serum and purifying the antibodies against the antigen from the serum. These Ab are specific for the desired antigen/hormone.

For the assay, a synthetic analog of the peptide hormone is synthesized such that the specific antibody recognizes and binds the synthetic analog as if it were the original complete antigen. The use of a synthetic analog by Iwasa et al. is important since the hormone/peptides in the bodily fluids are difficult and expensive to isolate, even in small quantities.

The synthetic artificial simulant peptide is then covalently coupled to a "reporter" indicator enzyme such as β-galactosidase (β-gal). B-gal hydrolyzes lactose into glucose and

galactose. It will also hydrolyze o-nitrophenyl-β-D-galactopyranoside to yield nitrophenol as a colored product.

The presence of the β-gal enzyme is determined by adding the o-nitrophenyl-β-D-galactopyranoside and, after time, looking for the colored product. Since the β-gal is coupled either to the antigen or its synthetic analog, the color formation indicates that the antigen is present.

The specific antibodies are immobilized (col. 6 line 53-) onto a solid substrate surface that could be glass, polystyrene beads, cellulose, etc. To this immobilized Ab surface is added the antigen/analog-enzyme complex (such as peptide-β-gal) such that the analog-β-gal complex occupies the binding sites of the immobilized antibodies. If o-nitrophenyl-β-D-galactopyranoside is added to the surface at this point, a brilliant blue-purple color develops and is used to indicate that the antibodies are bound and specific for the antigen.

In the actual detection of the antigen (hormone, etc) in bodily fluids (urine, blood, cerebrospinal fluid, etc) any antigen in the fluid added to the surface containing the Ab-analog-β-gal "sandwich" will compete for the binding sites occupied by the analog-β-gal complex on the Ab, displacing the simulant-indicator enzyme into the medium. The medium (solution) is recovered from the surface and o-nitrophenyl-β-D-galactopyranoside is added; the intensity (absorbance) of the resulting color is proportional to the amount of analog-β-gal displaced which is proportional to the amount of actual hormone (antigen) present in the bodily fluid sample. The hormone competes for the binding site on the specific Ab displacing the analog-enzyme complex. Alternatively, one could measure the decrease in absorbance of the immobilized

surface after exposure to the sample and washing away the displaced analog/labeling protein complex.

The Claimed Invention Distinguished from Iwasa et al.

Claim 1, and by extension claims 2-4 which depend therefrom, are distinguishable from Iwasa et al. for at least the following reasons:

a. "*obtaining a colorimetric indicator that has been reversibly incorporated into a binding protein, said binding protein having an active site at which the analyte will bind if present, said colorimetric indicator being reversibly bound at said active site*"

The examiner contends that Iwasa et al. disclose a method of real-time testing for the presence of an analyte including the above noted step, citing col. 9, line 65 - col. 10, line 15, wherein it is stated:

> The labeling enzyme is desirably an enzyme which is stable and has a high specific activity. Thus, there may be mentioned, for example, (1) carbohydrase [for example, glycosidase (e.g. .beta.-galactosidase, .beta.-glucosidase, .beta.-gluscuronidase, .beta.-fructosidase, .alpha.-galactosidase, .alpha.-glucosidase, .alpha.-mannosidase), amylase (e.g. .alpha.-amylase, .beta.-amylase, isoamylase, glucoamylase, Taka-amylase A), cellulase, lysozyme], (2) amidase (e.g. urease, asparaginase), (3) esterase [for example, cholinesterase (e.g. acetylcholinesterase), phosphatase (e.g. alkaline phosphatase), sulfatase, lipase], (4) nuclease (e.g. deoxyribonuclease, ribonuclease), (5) iron-porphyrin enzymes (e.g. catalase, peroxidase, cytochrome oxidase), (6) copper enzymes (e.g. tyrosinase, ascorbic acid oxidase), (7) dehydrogenase (e.g. alcohol dehydrogenase, malic acid dehydrogenase, lactic acid dehydrogenase, isocitric acid dehydrogenase), etc.

The enzymes listed by Iwasa et al. are not, however, colorimetric indicators. Except for the iron-porphyrin enzymes (col 10, lines 8-9), the enzymes themselves are <u>not</u> colored but react to yield a color if the appropriate substrate is used, as in the case of β-gal. The enzyme of Iwasa et al. does not change its spectral characteristics; the formation of a spectroscopically/fluorescently detectable product is the result of a subsequent chemical reaction

with appropriate added exogenous substrate such as o-nitrophenyl-β-D-galactopyranoside at a later stage. In contrast, the claimed colorimetric indicator is inherently colored and changes its optical properties on binding/unbinding.

Furthermore, it is incorrect to say that the Iwasa et al. enzyme is reversibly incorporated into the active site of the binding protein. The examiner appears to be confusing the binding protein on the surface (the Ab in Iwasa et al.) with the "labeling enzymes" listed in col. 9 line 67 - col 10, line 14. In Iwasa, the enzyme is attached to another molecule (eg. synthetic peptide/antigen, etc) that binds to the active site of the Ab. The enzyme itself does ***NOT*** bind at the active site/binding site. In the claimed invention, the indicator itself binds at the active/binding site of the binding protein, which is the site at which the analyte of interest will bind if present.

b. "*measuring at least one spectral value of said colorimetric indicator; and determining from any spectral value so measured said colorimetric indicator has been displaced from said binding protein and, thus, whether or not said analyte is present within said environment*"

In the presently claimed invention, the displacement of the colorimetric indicator (porphyrin, dye, etc) results in the immediate change in its spectral characteristics. There is no time-dependent, time-consuming incubation with an added enzyme substrate. The technology is "reagentless".

In Iwasa et al., the β-gal does not have a spectrum to change, and even if it did that change is not what is being measured. The spectral changes measured by Iwasa et al. are due to the relatively slow formation of a colored/fluorescent product (where there previously was no

color) due to subsequent and long-term (0.5 – 24 hours; col. 13, line 59) reaction. In Iwasa, the enzymes, even the heme-porphyrin indicators (col. 10, lines 8-9), do not change their spectral characteristics when the conjugated "peptide" binds/is released from the Ab nor are these monitored/measured to indicate the presence of the analyte in bodily fluids. Moreover, note that in Iwasa (col. 13, line 52) the competitive displacement of the labeling enzyme-peptide/analog complex occurs over a period of 1- 72 hours. The present invention as claimed allows for real time testing.

For at least these reasons it is respectfully submitted that Iwasa et al. does not anticipate claim 1.

With further reference to claim 3, it should be noted that, as previously explained, Iwasa et al. do not use porphyrin as a colorimetric indicator.

With further reference to claim 4, it should be noted that Iwasa et al. disclose (col. 10, line 6) AChE as a "labeling enzyme" (col 9, line 65) NOT as a binding protein. Iwasa's binding protein is the Ab.

Addressing of Claim Rejections Under 35 USC 103

Claim 5, which depends indirectly from claim 1, stands rejected under 35 USC 103(a) as being unpatentable over Iwasa et al. in view of Khabashesku et al. The foregoing comments directed to Iwasa et al. are likewise applicable here. Khabashesku et al. was cited by the examiner for the purported teaching of the use of a microscope slide. In this respect, it should be noted that Khabashesku et al. (col. 4, line 66 - col. 5, line 7) indicate that the KBr pellets are placed on a microscope slide, not bound to it.

Claim 5 merely captures one potential embodiment of applicant's invention. The complex can be immobilized onto many different types of surfaces, including, as examples, fiberglass, cellulose, glass, quarts, gold, dialysis tubing and polystyrene.

Claims 6-8, which all depend directly or indirectly from claim 1, stand rejected under 103(a) as being unpatentable over Iwasa et al. in view of Chapoteau et al. Again, the foregoing comments directed to Iwasa et al. are likewise applicable here. Chapoteau et al. was cited as disclosing that when reactants form a complex or when a complex dissociates there is generally an absorbance shift to a different wavelength. The examiner took the teachings of Chapoteau and extended them to the Iwasa et al. method to conclude that it would have been obvious for Iwasa et al. to measure two spectral values, one being before exposure to the sample.

The change in wavelength of some compounds upon complex formation is indeed well known. However, Iwasa et al. are not measuring the wavelength shift of anything, let alone the labeling enzymes or peptide-enzyme complex. They are measuring the formation of p-nitrophenol generated by the activity of β-gal to hydrolyze o-nitrophenyl-β-D-galactopyranoside. This is not a wavelength shift, but the appearance of an absorbance at some wavelength where there was no absorbance at any wavelength due to the non-existence of any product at that point.

Applicant duly notes that different compounds have different absorbance spectra. Indeed, different colorimetric indicators that can be used in the present invention will have different spectra and each will have a specific change in the spectra upon association/dissociation with another molecule. However, claim 6 states that measurements are made at two spectral values, one corresponding to the wavelength of the indicator prior to and one after release from

the binding enzyme. Claim 7 further specifies the values to be measured at about 402 nm and 442nm. The reason for this is to get the specificity of the change - the wavelengths are specific for prior and post release - as well as the fact that the signal size changes (one goes up and one goes down). Further, by measuring the absorbance at the prior-to-exposure wavelength which will increase in light intensity (decrease in absorbance), "false" positive and false negative signals that could occur by the mere increase in absorbance (loss of light intensity) by dirt ,etc, etc is avoided. This aspect is not inherent in a system where only one wavelength is monitored. While the present invention is workable by measuring at one wavelength, the preferred schemes of claims 6-8 enhance the sensitivity and specificity and reduction in "false" readings.

* * * * *

Considering the foregoing, it is sincerely believed that this case is in a condition for allowance, which is respectfully requested.

This paper is intended to constitute a complete response to the outstanding Office Action. Please contact the undersigned if it appears that a portion of this response is missing or if there remain any additional matters to resolve. If the Examiner feels that processing of the application can be expedited in any respect by a personal conference, please consider this an invitation to contact the undersigned by phone.

Respectfully submitted,

1/5/04
DATE

SIGNATURE OF PRACTITIONER

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